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Frozen mysids as an alternative to live *Artemia* in culturing seahorses *Hippocampus abdominalis*

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Abstract

This investigation examined the effects on growth and survival of seahorses *Hippocampus abdominalis* Leeson 1827 fed a 25% body weight (wet weight) daily ration of live *Artemia* sp. enriched with Algamac-3050, frozen mysids *Amblyops kempi* or a combination of live enriched *Artemia* and frozen mysids. After 3 months there was no difference in seahorse length, wet weight, condition factor (CF), or food conversion ratios (FCR) between the treatments. Mean daily specific growth rate (SGR) was higher for the *Artemia*-only treatment than for the mysid-only treatment ($P < 0.05$). FCRs ranged from 6.14 g to 8.72 g dry weight of food required to give a 1-g dry weight increase in seahorses. There was no difference in survival between treatments. Fatty acid analysis revealed that mysids had a higher percentage composition of EPA, 20 : 5n-3, and DHA, 22 : 6n-3, but a lower composition of AA, 20 : 4n-6, than enriched *Artemia*. Percentage n-3 highly unsaturated fatty acids (HUFAs) in mysid levels were approximately twice that of *Artemia*. Proximate analysis revealed mysids to be higher than the enriched *Artemia* in protein and fats, and lower in water content. This experiment demonstrates that, although no growth advantage was derived from the use of frozen mysids, they can be used successfully as an alternative food to live enriched *Artemia* for *H. abdominalis*. The use of frozen mysids is highly recommended in commercial seahorse culture if the seahorses are to be sold into the live aquarium trade, as this may increase their chances of survival after sale.

Keywords: syngnathid, live food, frozen food, *Hippocampus abdominalis*

Introduction

Seahorses and other syngnathid fish are in demand for use as an ingredient in traditional Chinese medicine in a variety of south-east Asian countries and as aquarium fish and curios on a global scale (Marichamy, Lipton, Ganapathy, & Ramalingam 1993; Vincent 1995, 1996; Lourie, Vincent & Hall 1999). Declining catch in some overexploited wild stocks (Vincent 1996) has stimulated interest in commercial aquaculture of many seahorse species to supply these trades (Correa, Chung, & Manrique 1989; Anil, Kakati, Ganga & Zacharia 1999; Hilomen-Garcia 1999; Cartwright 2000; Forteath 2000; Halls 2000; Ignatius, Rathore, Jagadis, Kandasami & Victor 2000; Woods 2000a).

Seahorses use visual cues in predating live animals. Traditionally, maintaining seahorses has required the culture or collection of live prey organisms such as *Artemia*, mysid shrimp and copepods (Hilomen-Garcia 1999; Payne & Rippingale 2000; Woods 2000a). In commercial seahorse aquaculture, there is economic motivation to minimize the use of live food. Culture of live food items is generally expensive, and collection of wild live food is unreliable, may be restricted by legislation and may have deleterious effects on natural ecosystems. Therefore, any frozen or artificial food that will sustain the growth and high survival of seahorses will be attractive to the industry.

Mysid shrimp are commonly used by aquarium hobbyists, and sometimes commercial aquaculturists, to feed to seahorses (Garrick-Maidment 1997; Fenner 1998; Hilomen-Garcia 1999; Forteath 2000) because they appear to promote good growth and survival, and because some seahorse species can be

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weaned relatively easily onto frozen mysids. Mysid shrimp have also been shown to be important components of seahorse diet in the natural environment (Reid 1954; Lovett 1969; Tipton & Bell 1988; Woods 2002). However, there are few published reports that have qualitatively assessed the actual growth and survival benefits of feeding mysid shrimp (either live or frozen) to seahorses.

The big-bellied seahorse *Hippocampus abdominalis* Leeson 1827 is the focus of commercial aquaculture attempts in New Zealand and Australia, primarily for the live aquarium trade. A diet of enriched *Artemia* has been shown to support multiple generations of captive *H. abdominalis* with high survival (Woods 2000b, 2001). However, *Artemia* by themselves may not provide all the dietary requirements of the target animal (Watanabe, Oowa, Kitajima & Fujita 1978; Watanabe, Ohta, Kitajima & Fujita 1982; Barclay & Zeller 1996; Furuita, Konishi & Takeuchi 1999), and they can be costly to rear to the larger sizes required by larger animals. In addition, many aquarists who purchase seahorses are restricted to providing them with non-live food. Therefore, survival of seahorses should be enhanced if they are accustomed to non-live foods before they are sold through the aquarium trade.

In an earlier investigation involving *H. abdominalis* (Woods 2003), 1- to 2-month-old juveniles were successfully weaned onto both frozen (Cyclop-Eeze copepods; Argent Laboratories, USA) and artificial (Golden Pearls; Brine Shrimp Direct, USA) foods, although growth was generally not as good as on enriched *Artemia*. This study indicated the potential to explore non-live food alternatives in the culture of *H. abdominalis*. The aim of this investigation is to determine the efficacy of using frozen mysids to rear cultured *H. abdominalis*.

Materials and methods

Ninety captive-bred F3 10-month-old *H. abdominalis* [mean $\pm$1 SE standard length (SL) = 113.6 $\pm$ 0.6 mm; mean wet weight = 2.92 $\pm$ 0.07 g; mean condition factor (CF) = 0.2 $\pm$ 0.003] were randomly selected from a captive population. These seahorses were randomly allocated to one of three treatments with a daily wet weight food ration of 25% of their wet body weight: fed live *Artemia* only, fed frozen mysids only or fed live *Artemia* and frozen mysids.

In the first feeding treatment, *Artemia* (GSL cysts from MacKay Marine Brine Shrimp Co., USA) that had been reared on a diet of 90% Eyre Peninsula Aquaculture brine shrimp food (proprietary rice bran-based product produced by Eyre Peninsula Aquaculture, Australia) and 10% *Spirulina* (Shengli Oilfield Group, China) to a suitable size for the juveniles were enriched for 24 h at a constant 28 °C using Algamac-3050 (Aquafauna Biomarine, USA). This regime and consequent enrichment with Algamac-3050 has been demonstrated to produce good growth and high survival in *H. abdominalis* (Woods 2003). After enrichment, *Artemia* were harvested and rinsed with fresh water before being used as food.

In the second treatment, the mysid *Amblyops kempi* (Holt & Tattersall, 1905) was used as food. These were the Hikari (Kyorin) brand of mysis shrimp, imported frozen and distributed by Biosuppliers, Auckland, New Zealand. The daily ration of frozen mysids required was thawed in sea water before being fed to the seahorses.

In the third feeding treatment, the *Artemia*+mysid treatment, the 25% body weight ration was split 50 : 50 between *Artemia* and mysids.

Tanks used were 9-L transparent plastic flat-bottomed circular fish bowls (28 cm in diameter, 15 cm in height). Sea water heated to a constant 18 °C and filtered to 20 μm entered each tank down a central inflow line to the bottom of the tank and exited through a 360° spray nozzle (Plassay® microjet garden spray) (see Woods 2003). This created a current from the central inflow line and across the tank bottom, with a gradual weakening of the current up the tank sides and back down the central inflow line. Water flow through the tanks was $\approx$ 0.25 L min^{-1}. Individual strands of separated black shadecloth (1 mm diameter) attached to the base of the sea-water inflow line, and running to the water surface, provided holdfasts for the seahorses.

There were six replicate tanks randomly assigned per treatment, with five seahorses per tank. There were no significant differences in seahorse length, weight or CF between treatments or replicates at the start of the experiment (ANOVA, $P > 0.05$). CF was calculated for seahorses as follows: CF = [wet weight (g)/length (cm^{-3})] $\times$ 100. Sex ratio of the seahorses used for the experiment was 50 : 50, with three tanks in each treatment with two males–three females, and the other three tanks with three males–two females. Seahorses were fed once at approximately 10.00 hours each day. Uneaten food and faeces were siphoned from each tank $\approx$ 6 h later to prevent tank fouling.

Monthly censuses were conducted to measure growth (length and wet weight). Length was

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measured to the nearest mm by placing seahorses on a plastic ruler. Length was measured according to the method of Lourie *et al.* (1999). Wet weight was measured on a Mettler P440 balance after blot-drying of seahorses. Wet weights were conducted 16 h after the last access of seahorses to food. After each census, the daily wet weight of each diet was adjusted according to average wet weight increase for each treatment to maintain the 25% ration (i.e. monthly ration adjustment).

A photoperiod of 12-h L : 12-h D was provided by timer-controlled 2×58 W cool white fluorescent tubes above the tanks at a mean intensity of 663.7 ± 22.3 lux. Tanks were inspected daily and uneaten food and faeces siphoned. Tanks were completely drained and cleaned every week with chlorine and detergent.

If mortalities occurred, the bodies were removed. In order to prevent potential increased growth rates in seahorses with increased access to food through the loss of competing tank-mates, dead seahorses were replaced with identifiable (i.e. unusually slender bodies or long snouts) seahorses of the same size in order that original stocking densities were maintained. Replacement seahorses were not included in final analyses.

Each week, any uneaten mysids and *Artemia* from one day's feed ration were siphoned from three tanks in each treatment at 15.00 hours. The wet weight of these mysids and *Artemia* was recorded, and they were then placed on preweighed filter papers and dried at 60 °C for 24 h. Dry weight measurements were used to calculate the proportion of food being offered that was consumed in relation to the calculated dry weight of seahorses (based on dry weight = 24% of wet weight of *H. abdominalis* if dried at 60 °C for 48 h; Woods 2002) via the calculation of FCRs as follows: CC/(Wf–Wi), where CC = mean cumulative dry weight of food consumed, Wf = mean final seahorse calculated dry weight per replicate tank, and Wi = mean initial seahorse calculated dry weight per replicate tank.

The experiment was concluded after 90 days. Final seahorse length and wet weight were recorded. Mean specific growth rate (SGR) for each tank's seahorses was calculated (SGR% increase in body weight day^{-1}) = [(lnWf–lnWi)/t] × 100, where Wf = final wet weight, Wi = initial wet weight, and t = number of days.

Mean water parameters during the experiment were: temperature (18.3 ± 0.01 °C), dissolved oxygen (7.6 ± 0.1 mg L^{-1}), salinity (34 ± 0.1 p.p.t.) and pH (8.1 ± 0.01).

Fatty acid analysis

Fatty acid analyses were performed by a commercial analytical laboratory. Triplicate samples of *Artemia* enriched for 24 h at 28 °C on Algamac-3050 and triplicate samples of the frozen mysids were stored over dry ice to ensure that they were well frozen. The samples were then freeze-dried for 2 days. Total dry weights were recorded, then samples taken for lipid extraction.

Samples of freeze-dried material (83–129 mg) were weighed into 8-mL test tubes. Methanol (1 mL) and dichloromethane (2 mL) were added, the test tubes were shaken on a vortex mixer and then placed in an ultrasonic bath for 5 min. Each tube was briefly centrifuged, and the solvent was transferred by Pasteur pipette to a second tube. This procedure was repeated once more.

The solvent ($\approx$ 6 mL) was washed with 1.5 mL of KCl solution (0.88% in water). The two phases did not separate cleanly, so the tube was centrifuged to break the emulsion. The upper layer (containing non-lipid material) was removed by Pasteur pipette and discarded. The lower layer was washed with 1.5 mL of methanol–water (1 : 1). The solvent was removed under a stream of argon at 35 °C. Final traces of solvent were removed on the freeze drier. The weight of the lipid extract was then recorded.

Fatty acids in the oil (free and bound) were converted to methyl esters with H_2SO_4 in methanol. Petroleum ether (0.5 mL containing the internal standard) and 1% H_2SO_4 in methanol (1 mL) were added to the lipid extract, the test tube was flushed with argon, sealed, then placed in a water bath at 50 °C overnight. Salt solution (NaCl 5%, 2 mL) was then added, and the fatty acid methyl esters (FAME) were extracted into 2×2 mL petroleum ether. The upper phase was removed and washed with sodium bicarbonate solution (2%, 2 mL). Gas chromatographic (GC) analysis was performed on an HP-5890 gas chromatograph using an Alltech EC-Wax column (0.25 mm × 30 m). The temperature programme was 165 °C for 3 min, 4 °C min^{-1} to 195 °C for 10 min, 4 °C min^{-1} to 225 °C for 12 min. Peaks were identified by comparison with cod liver oil used as an internal standard. Nonadecanoic acid (19 : 0) was used as an internal standard.

Proximate analysis

Analyses (protein, fat, ash and moisture) were performed by a commercial analytical laboratory on

frozen 100-g samples of the *Artemia* enriched for 24 h at 28 °C on Algamac-3050, and frozen mysids. Analysis was conducted on duplicate subsamples from the 100-g mass. Methods were as follows: ash [JAOAC 45, 548 (1962)] and AOAC (1990), for fat (methods 922.06, 948.15), protein (method 968.06) and moisture (method 930.15).

Data analysis

Statistical analyses were conducted using NCSS 2000 (Number Crunching Statistical Systems, Kaysville, UT, USA). Differences in standard length, wet weight and CF were tested using nested ANOVA with post hoc Student–Neuman–Keul's (SNK) ($P = 0.05$). Differences in mean percentage survival (arcsine-square root transformed), SGR and FCR for replicate tanks were tested using Kruskal–Wallis ANOVA with post hoc SNK ($P = 0.05$).

Results

After 3 months there were no significant differences in seahorse length, wet weight or CF between the treatments ($P > 0.05$) (Table 1). As a secondary test, Kruskal–Wallis ANOVAs ($P = 0.05$) were performed on the mean differences in initial and final lengths, wet weights and CFs per replicate tank for each treatment to determine whether there were any proportional differences; there were no significant differences.

Comparison of mean daily SGR revealed significant differences between the treatments ($H_{2,17} = 7.28$, $P < 0.05$), with seahorses in the *Artemia*-only treatment having a higher SGR than the mysid-only treatment, but not the *Artemia*+mysid treatment ($P < 0.05$).

Collection of uneaten food over the course of the experiment revealed that all *Artemia* presented to the seahorses were consumed within the 6-h feeding period, in both the *Artemia*-only treatment and the *Artemia*+mysid treatment. In the mysid-only treatment, mean percentage consumption was 54.5 ± 3.1%, whereas in the *Artemia*+mysid treatment, mean percentage consumption of mysids was 48 ± 4.2%.

Calculation of mean FCR revealed no significant difference between the three treatments ($P > 0.05$) with FCRs ranging from 6.14 g to 8.72 g dry weight of food required to give 1 g dry weight increase in seahorses, although the FCR in the mysid-only treatment appeared to be slightly higher (Table 1).

In terms of survival, there were no differences in percentage survival ($P > 0.05$), with only one mortality in the *Artemia*+mysid treatment. One replicate tank (five seahorses) in the mysid-only treatment was lost in the third month of the experiment when the water inflow line was accidentally knocked out overnight. As this was not related to the experimental treatments, this loss was not included in the survival analysis.

Fatty acid analysis

Marked differences in fatty acid profiles were observed between the *Artemia* and mysids (Table 2). Mysids had a higher percentage composition of both EPA, 20:5n-3, and DHA, 22:6n-3, but a lower composition of AA, 20:4n-6. Percentage n-3 highly unsaturated fatty acids (HUFAs) in mysid levels were approximately twice that of *Artemia*. Both DHA : EPA

Table 1 Initial and final measurements of length, wet weight, condition factor (CF), percentage survival, mean specific growth rate (SGR) and mean food conversion ratio (FCR) of *Hippocampus abdominalis* fed *Artemia* only, frozen mysids only or *Artemia* + frozen mysids at 25% body weight daily ration

	***Artemia* only**	**Mysids only**	***Artemia*+mysids**
Initial length (mm)	113.1 ± 1.0	113.0 ± 1.1	114.6 ± 1.2
Final length (mm)	138.4 ± 1.4	133.6 ± 5.2	135.8 ± 4.8
Initial weight (g)	2.75 ± 0.08	2.99 ± 0.11	3.03 ± 0.14
Final weight (g)	6.22 ± 0.28	5.3 ± 0.36	5.62 ± 0.36
Initial CF	0.19 ± 0.01	0.21 ± 0.01	0.2 ± 0.01
Final CF	0.23 ± 0.01	0.22 ± 0.01	0.2 ± 0.01
% survival	100	100	96.7 ± 3.7
SGR	4.14 ± 0.25^{a}	2.77 ± 0.27^{b}	3.33 ± 0.25^{ab}
FCR	6.14:1	8.72:1	6.9:1

Rows with different superscripts are significantly different ($P < 0.05$).

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Table 2 Fatty acid composition (%) of *Artemia* enriched for 24 h at 28 °C on Algamac-3050, and frozen mysids

Fatty acid	*Artemia*	Mysids
14:0	2.5±0.38	2.28±0.09
16:0	16.43±1.16	23.97±0.2
16:1 n-9	0.53±0.08	0
16:1 n-7	2.32±0.4	4.15±0.06
18:0	6.14±0.12	2.51±0.02
18:1 n-9	16.26±2.74	7.12±0.09
18:1 n-7	5.08±0.33	3.59±0.06
18:2 n-6	9.82±2.68	1.18±0.03
18:3 n-3	0.65±0.13	1.41±0.04
20:4 n-6	5.56±0.91	0.84±0.01
20:5 n-3	10.56±1.41	22.6±0.19
22:5 n-3	0.61±0.1	0.68±0.01
22:6 n-3	12.82±1.24	22.76±0.14
DHA:EPA	1.21	1.01
Oleic acid:DHA	1.27	0.31
EPA/AA	1.9	26.9
% known	90.74±0.46	96.17±0.05
% n-3 HUFA	25.66±2.18	49.07±0.3
%PUFA	41.04±0.5	51.29±0.29
%MUFA	24.36±2.43	15.73±0.11
%SFA	25.34±1.63	29.15±0.42
% lipid DW	8.89	9.13
% lipid WW	0.53	0.75

Data are the means±1 SE of three samples.

and oleic acid : DHA ratios were higher in enriched *Artemia*, although their EPA : AA ratios were lower than that of the mysids, which were particularly high.

Proximate analysis

Mysids had a higher percentage composition of proteins and fats than the enriched *Artemia* (Table 3), with a lower moisture composition.

Table 3 Proximate composition (%) of *Artemia* enriched for 24 h at 28 °C on Algamac-3050, and frozen mysids

	Artemia	Mysids
Protein	3.9	6.5
Fat	0.77	0.86
Moisture	93.3	88.9
Ash	1.7	3.1

Data are the means of duplicate subsamples.

Discussion

In this study, feeding live enriched *Artemia* only, frozen mysids only or a combination of the two did not result in significant differences in seahorse length or weight increase, although SGR was higher in the *Artemia*-only treatment. In addition, there were no differences in survival, with almost 100% survival being recorded across the three treatments. This demonstrates that frozen mysids can be used as food in rearing *H. abdominalis*.

However, there is a larger issue to consider other than demonstrating the usefulness of frozen mysids in promoting good growth rates and high survival in rearing seahorses – that of the ability to wean seahorses onto non-live foods. This ability to wean seahorses onto non-live foods is of vital importance to economically feasible and responsible seahorse culture for two main reasons.

First, the use of non-live foods either partially or wholly as a substitute for live foods could significantly reduce culture costs by mitigating the plant space, materials and labour costs involved in producing live foods. However, this is highly dependent upon a number of factors such as price paid for the frozen foods, cost of labour, cost of *Artemia* diets and efficiency of bulk live food-producing units. Also, in comparison with live food culture, which always carries some degree of uncertainty and risk in terms of the reliability of sustained production, the storage and use of frozen foods provides the culturist with a more reliable and known quantity and quality of food for seahorse production. Even if seahorses can be weaned entirely to a non-live diet, it is also still recommended to add some live feed component to the diet to maintain a healthy interest in feeding by seahorses, which may otherwise start to exhibit a decreased interest in feeding (Giwojna 1990).

Secondly, the ability to wean cultured seahorses on to non-live foods before sale in the aquarium trade should significantly improve their chances of survival. Many aquarium owners who purchase seahorses do not have the time, inclination or resources to culture reliably sufficient quantities of their own live food. They may also not be able to access or afford to buy live foods from commercial retailers in the quantities that maintaining healthy seahorses requires. The improved chance of survival derived from selling seahorses weaned onto non-live foods may also have the beneficial flow-on effect of reducing repeat purchasing of new seahorses to replace those that have not been able to be reared successfully in captivity after sale, many of which are currently sourced from wild populations that are being exploited in a seemingly unsustainable manner (Vincent 1996).

Because of their size, commercially available frozen mysids such as those used in this investigation are not suitable for feeding whole to early juvenile seahorses. The mysids we used were ≈ 10 mm in length and are readily accepted by general stock *H. abdominalis* as small as 80–90 mm SL, but are too large for seahorses smaller than this to ingest. The feeding of live prey such as enriched *Artemia* nauplii to newborn *H. abdominalis* is still an essential practice in the culture of this species, as newborn *H. abdominalis* find it very difficult to feed on non-live diets that require moving water currents to keep the food in suspension (Woods 2003). As they increase in size and become stronger swimmers and practised at food capture, juvenile *H. abdominalis* can be fed successfully on non-live diets (i.e. at 1 and 2 months of age; Woods in press) smaller than the frozen mysids we used in this investigation.

According to Sargent, McEvoy & Bell (1997), fish naturally contain high levels of the HUFAs docosahexaenoic acid (DHA), 22:6(n-3), and eicosapentaenoic acid (EPA), 20:5(n-3). Because of this, they have high nutritional requirements for these fatty acids, as well as for other fatty acids such as arachidonic acid (AA), 20:4(n-6), and linolenic acid, 18:3(n-3), which are termed essential fatty acids (EFAs). In this study, the highest EPA and DHA percentages were found in the frozen mysids, which also contained the highest percentage of n-3 HUFAs. However, these higher percentages of EFAs in the mysid shrimp did not result in increased growth rates.

Although there were no differences in growth rate in seahorses fed *Artemia* only or a combination of *Artemia* and frozen mysids, there may be physiological benefits from mixed feeding. For example, in broodstock nutrition, lipids are critically important sources of metabolic energy in gonad formation and in the formation of cell and tissue membranes (Watanabe, Arakawa, Kitajima & Fujita 1984; Sargent 1995). Hilomen-Garcia (1999) reported that established mating pairs of captive seahorses (*Hippocampus kuda*) fed a combination diet of HUFA-enriched *Artemia* (at 15% body weight) and mysids (at 6% body weight) or tilapia fry (at 5% body weight) showed more parturition events and greater brood size than those fed on a HUFA-enriched *Artemia*-only diet (at 30% of body weight). Thus, mixed feeding of the enriched *Artemia* and frozen mysids, such as used in this study, with their quite different HUFA profiles could well be a beneficial practice.

In other cultured finfish species that have been researched extensively, FCRs on non-live diets have become very efficient. For example, Webster, Thompson, Morgan, Grisby & Dasgupta (2001) obtained an FCR of 1.7 in hybrid sunshine bass fed once a day to satiation on a commercial pellet diet (EXT 400). Although not grossly inefficient, the FCRs we recorded for *H. abdominalis* (from 6.14:1 to 8.72:1) should be targeted for improvement. As seahorses are likely to be inefficient feeders because of their associated feeding morphology (they lack masticatory structures and a true stomach), the impact of feeding rate and regime on cultured seahorse FCR should be investigated further.

Optimal feeding rates (i.e. what percentage body weight per day to feed) and regimes (i.e. how many times to feed each day) have not yet been determined for most cultured seahorse species. In order to maximize economic benefit, it is imperative to determine the point at which the least amount of food fed to seahorses results in the most efficient conversion to growth (i.e. maximize FCR). Feeding past this point is economically wasteful. It may be that the feed rate of 25% body weight, as used in this investigation, represented overfeeding, with inefficient food conversion; lower feeding rates may improve FCRs. The fact that only ≈ 50% of the mysids offered in this experiment were actually consumed indicates that a more appropriate body weight ration with this food might be closer to 10–15%.

In conclusion, this study has demonstrated the effective use of frozen mysids as an alternative to live enriched *Artemia* in *H. abdominalis*, not only in terms of promoting growth and high survival, but also as a means of potentially increasing the efficiency of culture economics and increasing seahorse survival after sale in the aquarium trade. However, there were no improved growth benefits in using the frozen mysids over the enriched *Artemia*. The next logical steps are to determine optimal feeding rates (i.e. how much body weight) and regimes (i.e. how often) of seahorses on frozen mysids alone or in combination with other foods, and to determine how to improve further the nutritional value of frozen mysids in relation to dietary requirements of seahorses in order that growth performance is significantly improved over the use of live foods.

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